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(71) Applicant : TAKEDA CHEMICAL INDUSTRIES, LTD.
1-1, Doshomachi 4-chome
Chuo-ku, OSAKA (JP)

(72) Inventor : Ootsu, Koichiro
9-26, Todaiji 3-chome, Shimamoto-cho
Mishima-gun, Osaka 618 (JP)

(74) Representative : Laredo, Jack Joseph
Elkington and Fife Prospect House 8
Pembroke Road
Sevenoaks, Kent TN13 1XR (GB)

(54) Immunostimulant agent containing interleukin-2 and 5'-deoxy-5-fluorouridine.

(57) Disclosed is an immunostimulant agent containing interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof in combination, which shows a strong therapeutic effect by synergistic action and weak side effects. The immunostimulant agent may further contain another chemotherapeutic agent and/or another immunotherapeutic agent.

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BACKGROUND OF THE INVENTION

The present invention relates to an immunostimulant agent, and more particularly to a clinically applicabl immunostimulant agent containing interleukin-2 (hereinafter also briefly referred to as IL-2) and 5'-deoxy-5-fluorouridine (generic name: deoxifluridine, hereinafter also briefly referred to as 5'-DFUR).

Attempts have been made in recent years, as immunostimulant agent and various viral infections, by using the so-called lymphokines such as IL-2 for immunopotentiation [J. Immunol., 125, 1904 (1980)]. Recently, IL-2 obtained by genetic engineering techniques has been known (Japanese Patent Unexamined Publication Nos. 60-115528/1985 and 61-78799/1986).

On the other hand, 5'-DFUR was synthesized in 1979, and the effectiveness thereof in clinical tests was discovered [Cancer and Chemotherapy 12(1), 2044 (1985)]. For this reason, 5'-DFUR has recently been on the market.

At present, the treament of cancer has been attempted by operative therapy, radiotherapy and hormonotherapy, which are effective against primary cancer. However, metastasized cancer and cancer which has been discovered too late cannot be so treated, therefore, pharmacotherapy is used. Anticancer drugs currently usable exhibit a useful effect, but have strong side effects on organisms. Pharmacotherapy is not completely satisfactory as therapy for patients.

In recent years, lymphokines such as IL-2 have been used as antitumor agents to treat human malignant tumors [Cancer and Chemotherapy 13, 977 (1986)], and therapeutic effects thereof have also been reported [New England J. Med. 316, 889 (1987)].

SUMMARY OF THE INVENTION

The present inventors have hitherto conducted various investigations into the therapy of administering immunotherapeutic agents in combination with chemotherapeutic agents and IL-2 to enhance immunostimulant effects on malignant neoplasms. As a result, the present inventors discovered the fact that the administration of 5'-DFUR in combination with IL-2 to cancer-carrying animals exhibits each function co-operatively and gives a strong therapeutic effect.

The present invention provides:

(1) in one embodiment, a method for immunostimulating a mammal, which comprises administering an effective amount of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof,

(2) in another embodiment, a pharmaceutical composition for immunostimulating a mammal which comprises an effective amount of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof in a pharmaceutical carrier,

(3) in another embodiment, the combination of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof for the manufacture of a medicament for immunostimulating a mammal,

(4) in still another embodiment, a method for producing a pharmaceutical composition for immunostimulating a mammal, which comprises using interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof, and

(5) in still another embodiment, a kit of pharmaceutical preparations for immunostimulating a mammal, which comprises a pharmaceutical preparation of interleukin-2 and a pharmaceutical preparation of 5'-deoxy-5-fluorouridine.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As the above-mentioned IL-2, any substance may be used, as long as it has IL-2 activity, namely the activity of being able to promote and maintain in vitro long-term cultures of T cells while keeping their functions.

Examples of such substances include natural IL-2 produced in animal bodies or animal cells, IL-2 produced by recombinant technology and their related substances. When the IL-2 described above and the related substances thereof are proteins, they may have sugar chains or not.

Specific xamples thereof include polypeptide (I) (human IL-2) having the amino acid sequence shown in SEQ ID NO:1 (SEQ ID NO: 1) and a fragment having a porti n of the amino acid sequence necessary for its biological or immunological activity. Examples of th above-mentioned fragm nts include a fragment lacking one amino acid residue at the amino terminus (European Patent Publication No. 91539), a fragm nt lacking 4 amino acid residues at th amino terminal portion (Japan se Patent Unexamined Publication No. 60-126088/1985) and a fragment lacking several amino acid residu s at the carboxyl terminal portion (Japanese Patent Unexamined Publication No. 60-126088/1985).

Further, a portion of the constituent amino acid residues of polyp ptide (I) having the amino acid sequence shown in SEQ ID NO:1 may be d l ted or substituted with a different amino acid residue(s). For example, the

cystine residue at the 125-position may be substituted with a serine residue (Japanes Patent Unexamined Publication No. 59-93093/1984 which corresponds to U.S. Patent No.4,518,584).

The above-mentioned IL-2 may be chemically modified, for example with a poly thylene glycol derivative (e.g. Japanese Patent Unexamined Publication No. 60-226821/1985 which corresponds to European Publication No. 154316).

In particular, human IL-2 produced by genetic engineering techniques and having the amino acid sequence shown in SEQ ID NO:1 is preferably used in the present invention. In this case, the human IL-2 may be a mixture of the IL-2 further having a methionine (Met) residue at its amino terminus and the IL-2 having no methionine as its amino terminus residue (Japanese Patent Unexamined Publication Nos. 60-115528/1985 which corresponds to European Publication No.145390 and 61-78799/1986 which corresponds to European Pulication No. 176299), or it may have no methionine residue at its amino terminus and start with an alanine (Ala) residue (Japanese Patent Unexamined Publication No. 61-78799/1986 which corresponds to European publication No. 176,299). Further, the IL-2 may have a sugar chain.

5'-DFUR is a known compound, which is described in J. Med. Chem. 22, 1330-1335 (1979), and produced by a method using 5-fluorouridine as a starting material as described therein.

5'-DFUR is known to have low toxicity to animals, and in particular its effects are reported in Cancer and Chemotherapy 15(5), 1747-1754 (1988).

IL-2 enhances the reactivity of lymphoid cells, thereby exhibiting its biological activity. It is therefore desirable that IL-2 is combined with an anticancer agent lower in immunosuppressive activity. 5'-DFUR is a drug fit for this purpose.

The IL-2 used in the present invention is low in toxicity. For example, even when human IL-2 having the amino acid sequence shown in SEQ ID NO:1 (which is obtained by separating a mixture of the IL-2 further having a methionine (Met) residue at its amino terminus and the IL-2 having no methionine residue at its amino terminus by an isoelectric focusing method similar to that described in Japanese Patent Unexamined Publication No. 61-78799/1986 which corresponds to European Publication No.176,299) is given intravenously to mice or rats in a dose of 10 mg/kg (1 mg = 3.5×10^4 units), no mice or no rats die of its toxicity.

5'-DFUR used in the present invention is low in toxicity as compared to other known chemotherapeutic agents. For example, even when 5'-DFUR is given to mice orally in a dose of 2,000 mg/kg or intraperitoneally in a dose of 500 mg/kg, no mice die. Also, when 5'-DFUR is given to organisms, it does not inhibit the activity of natural killer cells. In accordance with this invention, this compound, which does not inhibit the activity, is very suitable for administration in combination with IL-2.

Thus, the immunostimulant agents of the present invention are usually given orally or parenterally as pharmaceutical preparations containing these active ingredients and pharmaceutically acceptable carriers or excipients. For example, forms of the formulations include an aqueous solution in which each active ingredient is previously dissolved or a solid mixture obtained by lyophilization of each active ingredient or a mixture in which each solid is obtained by lyophilization of each solution containing each active ingredient or a combination of an aqueous solution in which one of the active ingredients is dissolved and a solid obtained by lyophilization of the other.

The immunostimulant agent of the present invention can be given as one preparation formulated by mixing these active ingredients and a pharmaceutically acceptable diluent, excipient, etc. if necessary, in accordance with pharmaceutical manufacturing methods known in the art. Further, the respective active agents are separately formulated using a pharmaceutically acceptable diluent, excipient, etc. if necessary, prepared as a kit of pharmaceutical preparations which comprises a pharmaceutical preparation of IL-2 and a pharmaceutical preparation of 5'-DFUR, and can be given as respective one preparation using a diluent, etc. when used. Furthermore, the respective active agents are separately formulated as described above, prepared as a kit of phamaceutical preparations and can be given to the same object separately, concurrently or at time intervals, through the same route or different routes. When the immunostimulant agents of the present invention are used in solution form, they are prepared by conventional methods, using solvents such as aqueous solvents (for example, distill d wat r), water-soluble solvents (for example, physiological saline and Ringer solution) and oil-soluble solv nts (for example, sesame oil and olive oil). Additiv s can be added such as solubilizing adjuvants (for example, sodium salicylate and sodium acetate), buffers (for example, sodium citrate and glycerin), isotonic agents (for example, glucose and invert sugar), stabiliz rs (for example, human serum albumin and polyethylene glycol), preservatives (for example, benzyl alcohol and phenol) and soothing agents (for example, benzalkonium chloride and procaine hydrochloride) if necessary.

The concentration of IL-2 in the solution is pref rably about 3 to about 500 mg/ml.

The concentration of 5'-DFUR in the solution is preferably about 10 to about 500 mg/ml.

Formulations for oral administration include, for xample, tablets, pills, granules, powders, capsules, syrups, emulsions and susp nsions. Such formulations are prepared by known m thods, and lactose, starch,

sucrose, magnesium stearate, etc. are used as the carriers or the excipients.

For parenteral administration, for example, injections and suppositories can be used. Examples of the injections which can be used include intravenous injections, subcutaneous injections, intradermic injections, intramuscular injections and drops. The injections are usually provided, with ampules charged with them. The suppositories for intrarectal administration are prepared by known methods.

When the immunostimulant agent of the present invention is formulated, it is desirable to add about 0.5 to 1% of human serum albumin (HSA) to prevent the activity of IL-2 from being lowered, as described in Japanese Patent Unexamined Publication No. 62-228026/1987 which corresponds to European Publication No.228,833. For example, a mixture of respective materials can be dissolved in distilled water or in physiological saline for injection.

The immunostimulant agents of the present invention are useful for treatment or prophylaxis of tumors of mammals such as mice, cats, dogs, cattle, horses, sheep, goats, rabbits and humans, and have a remarkable effect, for example, on apothanasia of mammals carrying tumors. Such subject diseases include leukemia of various kinds, malignant lymphoma, osteosarcoma, malignant melanoma, malignant choriocarcinoma, myosarcoma, ovary cancer, uterus cancer, prostate cancer, pancreatic carcinoma, cancer of digestive organs such as the stomach and the intestine, lung cancer, esophageal carcinoma, head and neck tumor and brain tumor.

When the formulations of the immunostimulant agents of the present invention are in solution form, such solutions are suitable for injection.

When the solid formulations obtained by lyophilization are used, they are dissolved in distilled water or physiological saline to use them as solutions for injection. The formulations may also be dissolved in solutions containing monosaccharides, sugar alcohols, amino acids, etc. as described above and pH adjusted as described above, if necessary, and then they may be used.

In giving the immunostimulant agents of the present invention, the amounts of IL-2 and 5'-DFUR used varies depending on the method for administration, the subject tumor, etc. However, 5'-DFUR is used preferably in an amount of about 0.1 to about 100 mg per 10 μg (350 units as IL-2 activity) of protein of IL-2, and more preferably in an amount of about 1 to 50 mg. IL-2 activity is assayed using a mouse cell strain which proliferates depending on the IL-2 concentration as described in Japanese Patent Unexamined Publication No. 60-115528/1985 which corresponds to European Publication No.145,390. The immunostimulant agents of the present invention can be given to mammals including humans orally or parenterally. The dosage of the immunostimulant agents of the present invention varies according to the kind of IL-2 used. For example, when the immunostimulant agents are given as injections, based on the protein amount of IL-2, it is preferred that they are given to mice in a dosage of about 0.1 to 500 μg and to mammals other than mice in a dosage of about 0.001 to 4 μg. When the immunostimulant agents are given as suppositories, drops and oral agents, the dosages are preferably about 0.01 to 20 μg/kg, about 0.001 to 2 μg/kg and about 0.2 to 4 μg/kg, respectively. On the other hand, when the immunostimulant agents are given as injections, based on the dosage (mg) of 5'-DFUR, they are given to mice, for example, in a dosage of about 1 to 500 mg/kg daily, and to mammals other than mice in a dosage of about 1 to 100 mg/kg daily.

For the immunostimulant agents of the present invention, IL-2 and 5'-DFUR separately formulated can be given to the same object concurrently or at time intervals. The time interval in this case may be, for example, about 12 to 24 hours, preferably about 3 to 9 hours and more preferably about 2 hours or less.

The immunostimulant agent of the present invention may further contain another chemotherapeutic agent and/or another immunotherapeutic agent. The chemotherapeutic agents include anticancer agents such as mitomycin, adriamycin, cisplatin, vindesine, vincristine, cyclophosphamide, ifosfamide, bleomycin, peplomycin and etoposide. The immunotherapeutic agents include microorganisms or bacterial cell wall skeletal components; immunologically active natural polysaccharides or cytokines obtained by genetic engineering technique; and colony stimulating factor. The above-mentioned immunologically active polysaccharides include lenthinan and schizophyllan. The bacterial cell wall skeletal components include muramyldipeptide derivatives, and the microorganisms include lactic acid bacteria. The natural cytokines or the cytokines obtained by genetic engineering technique include interferons.

When another chemotherapeutic agent and/or another immunotherapeutic agent is added to the immunostimulant agent of the present invention, it is used in an amount usually employed for treatment.

The combination of IL-2 and 5'-DFUR having low immunosuppressive activity provides the immunostimulant agents which have synergistic effect and weak side effects.

The agents of the present invention comprising IL-2 and 5'-DFUR exhibit a remarkable immunostimulant activity such as antitumor activity and macrophage activation activity which cannot be obtained by independent use of each component. It is preferable that the present immunostimulant agent is used for treating a mammal containing at least one tumor.

The present invention will hereinafter be described in detail with the following Experimental Examples and

Examples. It is understood of course that these Experimental Examples and Examples ar not intended to limit the scope of the invention.

The IL-2 used in the Experimental Examples and Examples is human IL-2 having the amino acid sequence shown in SEQ ID NO:1, namely IL-2 having the amino terminus starting with an alanine residue. The IL-2 is prepared by cultivating transformant E. coli N4830/pTB285 (IFO 14437, FERM BP-852) by a method similar to that described in Japanese Patent Unexamined Publication No. 61-78799/1986 which corresponds to European Publication No. 176299, highly purifying the cultivated product by a method similar to that described in Japanese Patent Unexamined Publication No. 60-115528/1985 which corresponds to European Publication No. 145390, and isolating the IL-2 by an isoelectric focusing method similar to that described in Japanese Patent Unexamined Publication No. 61-78799/1986 which corresponds to European Publication No. 176299. The specific activity thereof is about $5X10^4$ units/mg.

Transformant E. coli N4830/pTB285 described above was deposited with the Institute for Fermentation, Osaka, Japan (IFO) under the accession number IFO 14437 on April 25, 1985. This microorganism was also deposited with the Fermentation Research Institute, the Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, Japan (FRI) under the accession number FERM P-8199 on April 30, 1985. This deposit was converted to the deposit under the Budapest Treaty and the microorganism has been stored at FRI under the accession number BP-852.

Experimental Example 1

Comparative Experiment on Antitumor Activity of 5'-DFUR, IL-2 and Combination of 5'-DFUR and IL-2 to Subcutaneously Implanted Tumor

Tissue gruel (tumor cells ground by a homogenizer to a suspended state) of mouse colon carcinoma 26 (colic cancer No. 26) was subcutaneously implanted through an injection tube in sural regions of hind-limbs of male BALB/c mice each with a body weight of about 25 g. Twelve days after the tumor implantation, mice in which tumors had grown to a predetermined size were selected and divided into groups, and drug administration was initiated. IL-2 was subcutaneously given to the lateral abdominal region opposite to the tumor-implanted hind-limb once a day continuously for 14 days. IL-2 was dissolved in physiological saline (dissolving solution) containing 5% of normal mouse serum so that the resulting solution was given in an amount of 0.1 ml/20 g of body weight of mouse. 5'-DFUR was orally given to the mice once a day from the first day of the IL-2 administration for 14 days. 5'-DFUR was suspended in physiological saline so that the resulting solution was given in an amount of 0.2 ml/20 g of body weight of mouse. The antitumor effect was evaluated by measuring the weight of tumors 28 days after the tumor implantation, determining the average weight of the tumors of each experimental group, and determining the tumor weight ratio (T/C %) of the group of mice treated with the drug (T, 5 mice per group) to the group of mice untreated with the drug (C, 10 mice per group). The daily dosage of the drugs is shown by the weight of the drugs (IL-2: μg, 5'-DFUR: μg) per mouse. Experimental results are shown in Table 1.

Table 1

Dosage (μg/mouse/day) 5'-DFUR	IL-2	Number of mice	Tumor weight (mg) average±SD	Weight ratio of tumor (T/C%)	Increase in body weight(g) (12-22 days)
Untreated control		10	1105±130		2.2
0	20	5	532±151	48	1.8
1000	0	5	777± 12	70	2.2
1000	20	5	311± 74	28	0.9
2000	0	5	353±153	32	0.9
2000	20	5	55± 25	5	-1.7

Experimental Example 2

Comparative Experiment on Antitumor Activity of 5'-DFUR, IL-2 and Combination of 5'-DFUR and IL-2 to Subcutaneously Implanted Tumor

Tissue gruel of colic cancer No. 26 prepared similarly to Experimental Example 1 was subcutaneously implanted in lateral abdominal regions of female BALB/c mice each with a body weight of about 20 g by use of an injection tube. Seven days after the tumor implantation, mice in which tumors had grown to a predetermined size were selected and divided into groups, and drug administration was initiated. IL-2 was subcutaneously given to a lateral abdominal region opposite to the tumor-implanted sites once a day continuously for 15 days. 5'-DFUR was orally given to the mice once a day from the first day of the IL-2 administration, 14 times in total. IL-2 was dissolved in physiological saline (dissolving solution) containing 5% of normal mouse serum so that the resulting solution was given in an amount of 0.1 ml/20 g of body weight of mouse. 5'-DFUR was suspended in physiological saline so that the resulting solution was given in an amount of 0.2 ml/20 g of body weight of mouse. The antitumor effect was evaluated by measuring the weight of tumors 28 days after the tumor implantation, determining the average weight of the tumors of each experimental group, and determining the tumor weight ratio (T/C %) of the group of mice treated with the drug (T, 5 mice per group) to th group of mice untreated with the drug (C, 10 mice per group). The daily dosage of IL-2 is shown by the weight (μg) of the drug per mouse, and the daily dosage of 5'-DFUR is also shown by the weight (μg) of the drug per mouse. Results obtained by giving IL-2 alone and a combination of IL-2 and 5'-DFUR of the present invention are shown in Table 2.

Table 2

Dosage (μg/mouse/day) 5'-DFUR	IL-2	Number of mice	Tumor weight (mg) average±SD	Weight ratio of tumor (T/C%)	Increase in body weight(g) (7-21 days)
Untreated control		10	2382±331		2.4
0	20	5	2520±370	106	3.6
2000	0	5	1562±404	66	3.6
2000	20	5	123±147	5	1.2

Experimental Example 3

Examination of Tumor Activity to Subcutaneously Implanted Tumor by Combination of 5'-DFUR and IL-2 with Another Anticancer Agent

Tissue gruel of colic cancer No. 26 was subcutaneously implanted in abdominal regions of female BALB/c mice each with a body weight of about 20 g, in a similar manner to that of Experimental Example 2. 5'-DFUR and IL-2 were given once a day continuously 10 times from 14 days after the tumor implantation. 5'-DFUR was orally given, and IL-2 was subcutaneously given to the abdominal region opposite to the tumor-implanted site as in Experimental Example 2. Anticancer agents, mitomycin (hereinafter referred to as MMC), adriamycin (hereinafter referred to as ADR) and cyclophosphamide (hereinafter referred to as CPA), were intravenously given to the mice 7 and 10 days after the tumor implantation. The dosage of each drug is shown by the weight (μg) per mouse. Even when 5'-DFUR and IL-2 of the present invention were only given for 10 days after the tumors had grown, excellent effect could be exhibited by the combinations with the additional anticancer agents. Results (the tumor weight 28 days after the tumor implantation) are shown in Table 3.

Table 3

Dosage (μg/mouse/day) Anticancer agent		5'-DFUR	IL-2	Number of mice	Tumor weight (mg) average±SD
Untreated control				10	2320±783
	0(*)	0(**)	0(***)	5	1967±511
	0	2000	20	5	1138±224
MMC	60	0	0	5	1445±130
MMC	60	2000	20	5	275±210
ADR	100	0	0	5	1902±165
ADR	100	2000	20	5	171± 70
CPA	1000	0	0	5	1580±149
CPA	1000	2000	20	5	118±116

Table 3 (continued)

Dosage (μg/mouse/day) Anticancer agent		5'-DFUR	IL-2	Weight ratio of tumor (T/C%)	Increase in body weight (g) (14-28 days)
Untreated control					2.9
	0(*)	0(**)	0(***)	85	1.7
	0	2000	20	49	
MMC	60	0	0	62	1.7
MMC	60	2000	20	12	1.0
ADR	100	0	0	82	1.7
ADR	100	2000	20	7	1.5
CPA	1000	0	0	68	1.6
CPA	1000	2000	20	5	1.7

* Physiological saline was intravenously given in an

amount of 0.2 ml/20 g.

** Physiological saline was orally given in an amount of 0.2 ml/20 g.

*** The solvent (5% normal mouse serum-containing physiological saline) for IL-2 was subcutaneously given in an amount of 0.1 ml/ mouse.

Experimental Example 4

Comparative Experiment on Survival Effect of 5'-DFUR, IL-2 and Combination of 5'-DFUR and IL-2 to Subcutaneously Implanted Tumor

Tissue gruel of colic cancer No. 26 prepared similarly to Experimental Example 1 was subcutaneously implanted through an injection tube in femoral regions of male BALB/c mice each with a body weight of about 25 g. Twenty days after the tumor implantation, mice in which tumors had grown to a predetermined size were selected and divided into groups, and drug administration was initiated. IL-2 was subcutaneously given to lateral abdominal region opposite to the tumor-implanted site once a day continuously for 15 days. 5'-DFUR was orally given to the mice once a day from the day of the IL-2 administration, 15 times in total. IL-2 was dissolved in physiological saline (dissolving solution) containing 5% of normal mouse serum so that the resulting solution was given in an amount of 0.1 ml/20 g of body weight of mouse. 5'-DFUR was suspended in physiological saline so that the resulting solution was given in an amount of 0.2 ml/20 g of body weight of mouse. The antitumor effect was evaluated by observing the survival time of the tumor-carrying mice, determining the average (median) survival time of each experimental group, and determining the survival time ratio (T/C %) of the group of mice treated with the drug (T, 5 mice per group) to the group of mice untreated with the drug (C, 5 mice per group). The daily dosage of IL-2 is shown by the weight (μg) of the drug per mouse, and the daily dosage of 5'-DFUR is also shown by the weight (μg) of the drug per mouse. Results obtained by giving IL-2 alone and a combination of IL-2 and 5'-DFUR of the present invention are shown in Table 4.

Table 4

Drug	Dosage (μg/mouse)	Number of mice	Survival time (days)	average (median)	T/C (%)
Untreated control		5	33, 35, 35, 41, 56	35.5	
IL-2	10	5	41, 42, 43, 45, 56	43.5	123
5'-DFUR	2000	5	46, 52, 56, 69, 76	56.5	159
5'-DFUR + IL-2		5	85, 87, >93*, >93, >93	>92.8	>262

* >93: The mouse was slaughtered 93 days after the tumor implantation. The tumor completely disappeared.

Experimental Example 5

Comparative Experiment on Survival Effect of 5'-DFUR, IL-2 and Combination of 5'-DFUR and IL-2 to Subcutaneously Implanted Tumor by Different Schedule

Tissue gruel of colic cancer No. 26 prepared similarly to Experimental Example 1 was subcutaneously implanted through an injection tube in femoral regions of male BALB/c mice each with a body weight of about 25 g. Fourteen days after the tumor implantation, mice in which tumors grew to a predetermined size were selected and divided into groups, and drug administration was initiated. The subcutaneous administration of IL-2 to lateral abdominal region opposite to the tumor-implanted site was done once a day for 4 continuous days each week and was repeated for 5 weeks. The oral administration of 5'-DFUR to the mice is done once a day for 4 continuous days of each week starting on the first day of the IL-2 administration and was repeated for 5 weeks. IL-2 was dissolved in physiological saline (dissolving solution) containing 5% of normal mouse serum so that the resulting solution was given in an amount of 0.1 ml/20 g of body weight of mouse. 5'-DFUR was suspended in physiological saline so that the resulting solution was given in an amount of 0.2 ml/20 g of body weight of mouse. The antitumor effect was evaluated by observing the survival time of the tumor-carrying mice, determining the average (median) survival time of each experimental group, and determining the survival time ratio (T/C %) of the group of mice treated with the drug (T, 5 to 10 mice per group) to the group of mice untreated with the drug (C, 10 mice per group). The daily dosage of IL-2 is shown by the weight (μg) of the drug per mouse, and the daily dosage of 5'-DFUR is also shown by the weight (μg) of the drug per mouse. Results obtained by giving IL-2 alone, 5'-DFUR alone and a combination of IL-2 and 5'-DFUR of the present invention are shown in Table 5.

Table 5

Drug	Dosage (μg/mouse)	Number of mice	Survival time (days)	average (median)	T/C (%)
Untreated control		10	37, 38, 38, 40, 40, 37, 38, 38, 41, 48	38.4	
IL-2	20	5	41, 42, 50, 54, 55	50.5	132
5'-DFUR	2000	5	56, 59, 62, 63, 63	62.5	163
5'-DFUR + IL-2		10	65, 68, 85, 89, 97, 78, 79, 80, 83, 84	83.0	216

Experimental Example 6

Examination of Survival Effect to Subcutaneously Implanted Tumor by Combination of 5'-DFUR and IL-2 with Another Anticancer Agent

Tissue gruel of colic cancer No. 26 prepared similarly to Experimental Example 1 was subcutaneously implanted in femoral regions of femal BALB/c mice with a body weight of about 25 g. 5'-DFUR and IL-2 were given once a day for 4 continuous days each week starting on the 20th day aft r the tumor implantation. This administration was repeated for 4 w eks. 5'-DFUR was orally given, and IL-2 was subcutaneously giv n to abdominal region opposite to the tumor-implanted site as with Exp rimental Exampl 1. An anticancer ag nt, MMC, was intravenously given to the mice 7, 24, 31 and 38 days after the tumor implantation. The dosage of each drug is shown by the weight (μg) per mouse. Even when 5'-DFUR and IL-2 of the present inv ntion were given after the tumors had grown, excell nt survival effect could be exhibited by the combinations with the additional anticancer agent. Results (the experiment was repeated twice) are shown in Table 6.

Table 6

Drug	Dosage (μg/mouse)	Number of mice	Survival time (days)	average (median)	T/C (%)
Untreated control		5	35, 37, 37, 38, 39	37.5	
5'-DFUR + IL-2	2000 20	5	49, 54, 56, 59, 79	56.5	151
MMC + 5'-DFUR + IL-2	60 2000 20	5	65, 70, 79, 85, 88	79.5	212
Untreated control		5	34, 37, 40, 40, 46	40.0	
5'-DFUR + IL-2	2000 20	5	62, 62, 62, 75, 95	62.5	156
MMC + 5'-DFUR + IL-2	60 2000 20	5	76, 85, 91, 96, 96	91.5	229

Example 1

Preparation for injection:

IL-2	30 mg
5'-DFUR	6000 mg
Lactose	170 mg
HPC-L (oxypropyl cellulose)	10 mg
Total	6210 mg

The respective components were mixed at the above ratio, and then dissolved in distilled water for injection or in physiological saline. Human serum albumin (HSA) was added thereto to a concentration of 0.5%, followed by filtration using a membrane filter having a pore size of 0.22 μm. One ml portions of the resulting filtrate were dispensed into respective vial bottles and lyophilized to prepare an antitumor agent for injection. This preparation for injection is dissolved in 5 ml of distilled water for injection when using it.

Example 2

Preparation for injection:

IL-2	30 mg
5'-DFUR	6000 mg
Lactose	170 mg
Sodium lauryl sulfate	1000 mg
Total	7200 mg

The respective components were mixed at the above ratio, and then dissolved in distilled water for injection or in physiological saline. Human serum albumin (HSA) was added thereto to a concentration of 0.5%, followed by filtration using a membrane filter having a pore size of 0.22 μm. One ml portions of the resulting filtrate were dispensed into respective vial bottles and lyophilized to prepare an antitumor agent for injection. This preparation for injection is dissolved in 5 ml of distilled water for injection when using it.

Example 3

Preparation for injection:

IL-2	5 mg
5'-DFUR	60,000 mg
Lactose	200 mg
HPC-L (oxypropyl cellulose)	100 mg
Total	60,305 mg

The respective components were mixed at the above ratio, and then dissolved in 1000 ml of distilled water or physiological saline for injection. Human serum albumin (HSA) was added thereto to a concentration of 0.5%, followed by filtration using a membrane filter having a pore size of 0.22 μm. Ten ml portions of the resulting filtrate were dispensed into respective vial bottles and lyophilized to prepare an antitumor agent for injection. This preparation for injection is dissolved in 10 ml of distilled water for injection when using it.

Example 4

Preparation for injection:

IL-2	10 mg
5'-DFUR	120,000 mg
Lactose	200 mg
Sodium lauryl sulfate	1,000 mg
Total	121,210 mg

The respective components were mixed at the above ratio, and then dissolved in 1000 ml of distilled water or physiological saline for injection. Human serum albumin (HSA) was added thereto to a concentration of 0.5%, followed by filtration using a membrane filter having a pore size of 0.22 μm. Ten ml portions of the resulting filtrate were dispensed into respective vial bottles and lyophilized to prepare an antitumor agent for injection. This preparation for injection is dissolved in 10 ml of distilled water for injection when using it.

Example 5

Kit for injection preparation:

[A]	IL-2	10 mg
	Lactose	85 mg
	HPC-L (oxypropyl cellulose)	5 mg
	Total	100 mg

The three components were mixed at the above ratio, and then dissolved in 1000 ml of distilled water or physiological saline for injection. Human serum albumin (HSA) was added thereto to a concentration of 0.5%, followed by filtration using a membrane filter having a pore size of 0.22 μm. Five ml portions of the resulting filtrate were dispensed aseptically into respective vial bottles and lyophilized to prepare a kit A.

[B]	5'-DFUR	120,000 mg
	Sodium lauryl sulfate	20,000 mg
	Total	140,000 mg

The respective components were mixed at the above ratio, and then dissolved in 1000 ml of distilled water or physiological saline for injection, followed by filtration using a membrane filter having a pore size of 0.22 μm. Five ml portions of the resulting filtrate were dispensed aseptically into respective vial bottles and lyophilized to prepare a kit B.

[C] Distilled water for injection 10 mg

The kit A is dissolved with the kit C and then the kit B is dissolved therein to obtain a solution for injection when using the kit. Alternatively, the kit B is dissolved with the kit C and then the kit A is dissolved therein to obtain a solution for injection when using the kit.

Alternatively, the kit A and the kit B are dissolved with the kit C respectively, to prepare two solutions for injection, a kit A solution and a kit B solution, which are administered separately at the same time or at an interval.

Sequence Listing

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 133 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys
50 55 60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala

100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

Claims

1. Use of a combination of interleukin-2 with 5'-deoxy-5-fluorouridine or a salt thereof for the manufacture of a medicament for immunostimulating a mammal.

2. Use in accordance with claim 1, wherein the manufacture of the medicament comprises admixing interleukin-2 and 5'-deoxy-5-fluorouridine-or a salt thereof.

3. Use in accordance with claim 1, wherein the manufacture of the medicament comprises formulating separately interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof.

4. Use in accordance with claim 1, wherein the ratio of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof on the basis of 10 μg of protein of IL-2 is from about 0.1 to about 100 mg of 5'-deoxy-5-fluorouridine or a salt thereof.

5. Use in accordance with claim 4, wherein the ratio of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof on the basis of 10 μg of protein of IL-2 is from about 1 to about 50 mg of 5'-deoxy-5-fluorouridin or a salt thereof.

6. Use in accordance with claim 1, further containing other chemotherapeutic agent and/or another immunotherapeutic agent.

7. Use in accordance with claim 6, in which said chemotherapeutic agent is an antitumor agent.

8. Use in accordance with claim 7, in which said antitumor agent is selected from the group consisting of mitomycin, adriamycin, cisplatin, vindesine, vincristine, cyclophosphamide, ifosfamide, bleomycin, peplomycin and etoposide.

9. A pharmaceutical composition for immunostimulating a mammal which comprises an effective amount of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof in a pharmaceutical carrier.

10. A composition in accordance with claim 9, wherein the composition comprises mixing interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof.

11. A kit of pharmaceutical preparations for immunostimulating a mammal, which comprises a pharmaceutical preparation of interleukin-2 and a pharmaceutical preparation of 5'-deoxy-5-fluorouridine or a salt thereof.

12. A composition in accordance with claim 9, wherein the ratio of interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof on the basis of 10 μg of protein of IL-2 is from about 0.1 to about 100 mg of 5'-deoxy-5-fluorouridine or a salt thereof.

13. A composition in accordance with claim 9, further containing other chemotherapeutic agent and/or anoth r immunotherapeutic agent.

14. A composition in accordance with claim 13, in which said chemotherapeutic agent is an antitumor agent.

15. A composition in accordance with claim 14, in which said antitumor agent is selected from the group consisting of mitomycin, adriamycin, cisplatin, vind sine, vincristine, cyclophosphamide, ifosfamide, bleomycin, peplomycin and etoposide.

16. A composition in accordance with claim 9 wher in said interleukin-2 contains a biological or immunological active fragment.

Claims f r the following Contracting States : GR and ES

1. A method for producing a pharmaceutical composition for immunostimulating a mammal which comprises

an effective amount of interl ukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof in a pharmaceutical carrier, which comprises using interleukin-2 and 5'-deoxy-5-fluorouridine or a salt th reof.

2. A method in accordance with claim 1, wherein the method comprises mixing interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof.

3. A method in accordance with claim 1, wherein the method comprises formulating separately interl ukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof.

4. A method in accordance with claim 1, further comprises mixing other chemotherapeutic agent and/or other immunotherapeutic agent.

5. A method in accordance with claim 4, in which said chemotherapeutic agent is an antitumor agent.

6. A method in accordance with claim 5, in which said antitumor agent is selected from the group consisting of mitomycin, adriamycin, cisplatin, vindesine, vincristine, cyclophosphamide, ifosfamide, bleomycin, peplomycin and etoposide.

7. A method in accordance with claim 4, in which said immunotherapeutic agent is selected from the group consisting of microorganisms, bacterial cell wall skeletal components, immunologically active natural polysaccharides, cytokines obtained by genetic engineering technique and colony stimulating factors.

8. A method in accordance with claim 7, in which said immunologically active polysaccharide is lenthinan or schizophyllan.

9. A method in accordance with claim 7, in which said bacterial cell wall skeletal component is a muramyldipeptide derivative.

10. A method in accordance with claim 7, in which said microorganism is a lactic acid bacterium.

11. A method in accordance with claim 7, in which said cytokine is selected from natural cytokine and cytokine obtained by genetic engineering technique and is an interferon.

12. A method in accordance with claim 1 wherein said interleukin-2 contains a biological or immunological active fragment.

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(71) Applicant : Takeda Chemical Industries, Ltd.
1-1, Doshomachi 4-chome
Chuo-ku, OSAKA (JP)

(72) Inventor : Ootsu, Kolchiro
9-26, Todaiji 3-chome, Shimamoto-cho
Mishima-gun, Osaka 618 (JP)

(74) Representative : Laredo, Jack Joseph
Elkington and Fife Prospect House 8
Pembroke Road
Sevenoaks, Kent TN13 1XR (GB)

(54) Immunostimulant agent containing interleukin-2 and 5'-deoxy-5-fluorouridine.

(57) Disclosed is an immunostimulant agent containing interleukin-2 and 5'-deoxy-5-fluorouridine or a salt thereof in combination, which shows a strong therapeutic effect by synergistic action and weak side effects. The immunostimulant agent may further contain another chemotherapeutic agent and/or another immunotherapeutic agent.

EP 0 488 718 A3

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 91 31 0995

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	CANCER CHEMOTHERAPY vol. 8, 1986, pages 484 - 494 RÜMKE PH. 'Malignant melanoma' * page 491, line 17 - line 25 *	1-16	A61K39/39 A61K37/02
A	EP-A-0 089 062 (AJINOMOTO CO., INC) 21 September 1983 * claim 5 *	1-16	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5) A61K

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	07 JANUARY 1993	LEHERTE C.F.M.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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& : member of the same patent family, corresponding document

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